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Chemical profiling of essential oils: Investigations into modulating milk production in dairy cows using in silico methods

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Abstract: This study aimed to assess the biological and biotherapeutic activities of essential oils derived from the medicinal plants *Tanacetum vulgare* L., *Myrtus communis* L. subsp. communis L., and *Pimpinella flabellifolia* (Boiss.) Benth. Et Hook. ex Drude. Plant samples were systematically collected from the Sivas region of Türkiye. Subsequently, essential oils were extracted using a Clevenger-type apparatus, and their compositions were assessed by gas chromatography–mass spectrometry (GC-MS) analysis. Then, antioxidant activities of the essential oil samples were investigated using β-carotene-linoleic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Furthermore, the antimicrobial activity of these species was assessed via the disc diffusion assay. Finally, the potential effects of the essential oil compositions from these plants on milk production in dairy cows were analyzed through in-silico methods.

Introduction

The utilization of essential oils (EO) as supplements for animals, particularly dairy cows, has gained increasing attention in recent years due to their perceived potential to enhance various aspects of animal health and productivity (1). EO, extracted from aromatic plants, are known for their diverse bioactive compounds, which possess a range of biological and pharmacological activities such as antimicrobial, antioxidant, antifungal, and antibacterial (2). In the context of animal husbandry, these natural compounds are being explored as dietary supplements with the aim of enhancing overall well-being, mitigating stress, and potentially increasing products (3). Dairy cows, as vital contributors to the agricultural sector, are subject to various stressors and health challenges that can impact milk production. The incorporation of EO into their diet as a supplement represents a novel avenue for addressing these concerns (4). Türkiye is one of the world's most florally diverse countries. This distinction is attributed to the convergence of the Mediterranean, Iran-Turan, and Euro-Siberian phytogeographic regions within Anatolia, fostering dynamic interactions among plant communities (5). Furthermore, the considerable variations in climate, topography, geology, geomorphology, and aquatic environments play pivotal roles in shaping this botanical abundance. Sivas, a city in Türkiye, emerges as a province characterized by a rich floral spectrum, influenced by the distinctive geographical features and imprints of the Anatolian climate zone (6). Some of these plants analyzed in this study, collected from the Sivas region, not only highlights the local biodiversity but also unveiled the biotherapeutic potential inherent in the flora of this region.

The *Umbelliferae* (*Apiaceae*) family has a vast array of 300–455 genera and over 3000 species. Notably, anise (*Pimpinella anisum*) emerges as a crucial species employed for its aromatic qualities in both the food and perfumery industries. Beyond its industrial applications, *P. flabellifolia* holds significance in folk medicine, particularly for its beneficial effects, notably during lactation periods (7). Tepe et al. have documented that the EO of *P. flabellifolia* contains key biotherapeutic compounds, including limonene (47%), E-anethole (37.9%), and β-pinene (6%) (8). Furthermore, studies have identified E-anethole at 41% in the aerial parts and 63.5% in the fruits of *P. flabellifolia* (9). Recent research has highlighted the

utilization of certain Pimpinella species as supplements in animal feed, with a specific focus on enhancing milk secretion in dairy cows (10).

The Myrtaceae[Ma1] family includes 150 genera and approximately 3300 species, exhibiting a global distribution. Within this plant family, Myrtus communis is a key plant species renowned for its diverse pharmacological effects, including antioxidant, antimicrobial, and anti-inflammatory properties (11). EO composition of *M. communis* species cultivated in Türkiye has been documented, revealing constituents such as linalool (31.3%), linalyl acetate (17.8%), 1,8-cineole (14.7%), geranyl acetate (9.1%), α-pinene (8.4%), and α-terpineol (6.5%) (12). On the other hand, the genus Tanacetum encompasses over 200 species distributed across West Asia, North Africa, and Europe (13). Within Türkiye, there are 45 Tanacetum species and 18 of them are endemic. *T. vulgare*, a member of the Tanacetum taxa, is a crucial plant because of its diverse EO compositions and is recognized not only for its pharmacological effects but also for its application as a repellent against insects (14). The EO composition of *T. vulgare* includes camphor, borneol, β-thujone, and 1,8-cineole, albeit with varying percentages attributed to the specific collection area (15).

In this study, *P. flabellifolia*, *M. communis*, and *T. vulgare* were collected, and their EO compositions analyzed. Furthermore, diverse methods were employed to investigate the antioxidant and antimicrobial activities of these EOs. Lastly, in silico analyzes was performed to assess molecular mechanisms to examining the effects of EO supplementation on dairy cows, with a specific emphasis on its potential effects on milk production. Utilizing in silico molecular docking methods, our objective is to delineate the intricate interactions between EO compositions and crucial molecular targets associated with lactation and metabolic pathways. Through this investigation, we aim to provide valuable insights into the role of EO as dietary supplements, aiming to optimize the performance and well-being of dairy cows, potentially influencing both the quality and quantity of milk production.

Experimental Section

Plant Material Collections

The plant species collection regions are listed, *P. flabellifolia* from Sivas Gürün/Sivas, *M. communis* from Gürpınar/Sivas and *T. vulgare* from Ulaş-Tecer/Sivas. Voucher plant specimens were identified by Dr. Erol DONMEZ at the department of Biology, Cumhuriyet University, Sivas-Turkey and has been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher No: ED-11014 for *P. flabellifolia*, No: ED-11015 for *M. communis* and No: ED11005 for *T. vulgare*)

Isolation of Essential Oils

Air-dried and mechanically grinded aerial parts of all plant samples were distillated for 3 h using a Clevenger apparatus. Obtained oil samples were dried with anhydrous sodium sulphate, filtrated, and stored at +4°C.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

Chemical compositions of the EO samples were analyzed using a Shimadzu GC2010 GC–MS, equipped with a capillary column TC-5 (50 m× 0,32 mm i.d., 0.32 mm) and a 70 eV EI Quadrapol detector. Helium was the carrier gas, at a flow rate of 2,1 ml/min., injector and MS transfer line temperatures were set at 265 and 280°C, respectively. Column temperature was initially at 45°C held for 3 min, then gradually increased to 150°C at a 2°C/min rate, held for 4 min, and finally increased to 265°C at a 4°C/min and held for 4 min. Diluted samples (1:150 v/v, in hexane) of 0.5 µL were injected manually and splitless. The components were identified by comparison with their lineer retention indices (LRIs) and MS (NBS75K library data of the GC–MS system).

Antioxidant Activity Assays

Diphenylpicrylhydrazyl (DPPH) Assays

The methodology employed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the presence of a hydrogen-donating antioxidant. DPPH solutions exhibit a robust absorption band at 517 nm, manifesting as a deep violet color. As the absorption diminishes, the resultant decolorization is stoichiometrically linked to the extent of reduction. The residual DPPH, quantified after a specified duration, serves as an inverse measure of the radical scavenging activity exerted by the antioxidant. To execute this, 50 µl of various concentrations of the EOs were introduced to 5 ml of a 0.004% methanol solution of DPPH. Following a 30-minute incubation period at room temperature, absorbance readings were recorded at 517 nm against a blank. Inhibition free radical DPPH in percentage was calculated in the Equation 1.

$$DPPH\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad \textbf{Equation 1}$$

EO concentrations providing 50% inhibition (IC50) were calculated from the graph plotting inhibition percentage against varying concentrations. Synthetic antioxidant reagent butylated hydroxytoluene (BHT) was used as a positive control and all tests were carried out in triplicate.

β-Carotene-Linoleic Acid Assays

Antioxidant capacity was also determined by assessing

the inhibition of volatile organic compounds and conjugated diene hydroperoxides resulting from the oxidation of linoleic acid. This method relies on the alteration of the yellow color of β-carotene, attributed to its reaction with radicals generated during linoleic acid oxidation within an emulsion (16). The velocity of β-carotene bleaching is attenuated in the presence of antioxidants. This alteration serves as the basis for evaluating the antioxidant activity of the EO in comparison to well-established synthetic and natural antioxidants, such as BHT. The antioxidative capacities of the EOs were measured against BHT at equivalent concentrations, with a blank comprising only 350 µl of ethanol. All experiments were replicated three times for robustness and reliability.

Antimicrobial Activity Assays

The antimicrobial and antifungal activities of the EOs were systematically assessed against three Gram-positive bacteria, five Gram-negative bacteria, and one fungus. The bacterial and fungal strains utilized in this study were sourced from the Refik Saydam Hygiene Institute in Ankara, Turkey. Bacterial cultures were appropriately suspended in Mueller Hinton Agar (MHA-Oxoid-CM337), while yeasts were suspended in Sabouraud Dextrose Agar (OxoidCM41). To evaluate antimicrobial activity, a disc diffusion assay was performed (17). Initially, suspensions of the tested microorganisms (0.1 ml containing 10^8 cells per ml) were evenly spread on solid media plates. Subsequently, filter paper disks (6 mm in diameter), treated with 10 µl of EO, were placed on these plates. Then plates were incubated at 4°C for 2 hours and at 37°C for 24 hours for bacteria, and at 30°C for 48 hours for yeasts. The diameters of the resulting inhibition zones were measured in millimeters. All assays were performed triplicate.

In Silico Analysis

Molecular Docking

The use of computer-aided methodologies for drug and supplement development within the veterinary medicine domain has become a prominent focus in recent times. In silico experiments have been instrumental in elucidating specific molecular interactions between target protein structures and bioactive compounds within herbal mixtures. Prolactin, a hormone secreted by the pituitary gland, plays a pivotal role in milk production during pregnancy and postpartum in mammals (18). Increasing prolactin secretion in mammals has been identified as a strategy to enhance milk production, particularly in dairy cows (19). Various approaches in the literature decrease prolactin inhibitors, with dopamine being a notable example. Extensive studies have reported that the dopamine molecule and related processes act as prolactin inhibitors, thereby diminishing lactation (19). Similarly, literature suggests that elevated progesterone levels during pregnancy in mammals serve to prevent prenatal milk loss (20, 21).

From this knowledge, structures of dopamine (Uniprot ID: P20288) and progesterone (Uniprot ID: Q8MIL9) of the Bos taurus were selected as receptors in a semi-flexible molecular docking procedure (22). Molecular docking studies were conducted for ligands common in EO compositions of plants with the highest quantities. The in silico investigation employed Autodock Vina (23) and Discovery Studio Visualizer (24) programs for comprehensive assessment.

PASS Prediction

The PASS (Prediction of Activity Spectra for Substances) online web tool enables prediction of the expected biological function profile of a chemical compound with similarities to a drug (25). The compounds showing the lowest binding affinity and highest inhibitory potential from molecular docking analyses were further subjected to PASS analysis to reveal their other pharmacological effects. The PASS tool prediction results 2 category labels of “probability to be active” (Pa) or “probability to be inactive” (Pi) as biological activity.

Mutagenicity Prediction

Lazar, a web-based computational tool helps in the prediction of complicated toxicological properties such as toxicity, carcinogenicity, and blood brain barrier (BBB) permeation (26). Lazar employs data mining algorithms to input experimental data and generate predictions for unknown chemical ligands. For the analysis, the chemical SMILES data of phytochemicals were introduced to the system, and results were obtained by selecting the desired options for analysis.

Carcinogenicity Prediction

The potential of any chemical to induce carcinogenicity in humans or animals can be predicted computationally by using Carcinogenicity Prediction using Ensembled Learning Methods (CarcinoPred-EL). This web tool is created by combining different algorithms (RF, SVM and XGBoost), resulting in values with high sensitivity, accuracy, and specificity rates (27).

ADMET Analysis

ADMET (absorption, distribution, metabolism, excretion, and toxicity) analysis was performed by using the pkCSM (https://biosig.lab.uq.edu.au/pkcsm) web tool (28). For analysis, the SMILES chemical data format of the bioactive compounds was retrieved from the Pubchem database.

Statistical Analysis

The data were statistically analyzed by ANOVA (n=3) and statistical significance was accepted at a level of $p<0.05$.

Table 1. Chemical components of *Tanacetum vulgare* L., *Pimpinella flabellifolia* and *Myrutus communis* L.subsp .communis Essential Oils.

No.	LRI (cal)	LRI (lit)	Compound	Tanacetum vulgare L (%)	Pimpinella flabellifolia (%)	Myrutus communis L. subsp. communis (%)	IM
1	930	931	(-)-α-Pinene	3.02±0.03	5.1±0.03	14.94±0.05	a,b
2	953	954	camphene	1.48±0.02	nd	0.57±0.00	a
3	976	977	sabinene	2.35±0.04	nd	nd	a
4	977	979	β-pinene	nd	3.7±0.05	2.87±0.02	a
5	992	994	myrcene	nd	nd	0.4±0.02	a
6	1012	1010	β-Cymene	12.77±0.13	nd	nd	a
7	1013	1011	p-cymene	nd	1.6±0.03	nd	a,b
8	1014	1013	m-cymene	nd	nd	4.57±0.06	a
9	1031	1030	4-carene	nd	nd	1.35±0.01	a
10	1042	1041	ocimene	nd	nd	4.75±0.05	a,b
11	1050	1051	Thujan-4-ol	2.90±0.02	nd	nd	a
12	1062	1062	α-Terpinyl acetate	nd	nd	1.61±0.01	a
13	1065	1068	1,8-Cineole	5.15±0.04	nd	27.06±0.15	a,b
14	1081	1080	linalool	0.05±0.00	0.30±0.01	6.45±0.24	a,b
15	1086	1088	terpinolene	nd	0.80±0.03	2.53±0.03	a
16	1090	1091	β-Thujone	32.62±0.35	nd	nd	a,b
17	1117	1120	fenchol	nd	nd	0.35±0.02	a
18	1134	1133	(+)-cis-sabinol	0.23±0.01	nd	0.33±0.02	a
19	1145	1144	camphor	5.1±0.03	nd	nd	a,b
20	1161	1164	4-terpineol	1.49±0.01	1.45±0.03	5.28±0.04	a
21	1168	1165	thujanol	nd	nd	0.22±0.03	a
22	1182	1183	α -terpinene	nd	0.9±0.04	nd	a
23	1184	1185	myrtenol	nd	nd	3.14±0.05	a
24	1185	1186	α-terpineol	1.90±0.11	nd	nd	a
25	1202	1203	d-limonene	nd	34.2±0.23	5.25±0.08	a,b
26	1216	1218	β-phellandrene	nd	0.3±0.01	nd	a
27	1229	1227	anisaldehyde	nd	0.1±0.00	nd	a
28	1236	1235	linalyl acetate	nd	nd	0.93±0.10	a
29	1275	1276	sabinyl acetate	2.97±0.03	nd	nd	a
30	1284	1283	bornyl acetate	5.47±0.02	nd	nd	a
31	1286	1285	(E)-anethol	nd	48.30±0.24	nd	a,b
32	1297	1298	carvacrol	nd	0.30±0.00	2.42±0.01	a,b
33	1301	1299	myrtenyl acetate	nd	nd	3.25±0.06	a
34	1302	1306	thymol	nd	nd	3.85±0.03	a
35	1358	1360	geranyl acetate	nd	nd	1.07±0.01	a
36	1580	1578	spatulenol	nd	0.4±0.02	nd	a

Note: (a) Compounds listed in order of elution from a DB-5 column. (b) Identification of components based on standard compounds; All values are mean ± standard deviation of triplicates; LRI(cal): Linear retention indices (DB-5 column) calculated against n-alkanes. % calculated from FID data with standart; LRI(lit):https://pubchem. ncbi.nlm.nih.gov; IM: Identification Method; nd: not detected.

Results and Discussion

GCMS Analysis Results

The results of the volatile composition analysis of the EOs are presented in Table 1 and Figure 1. First, the EO samples extracted from T. vulgare, composed of mostly beta-thujone (32.62%), beta-cymene (12.77%), and bornyl-acetate (5.47%).

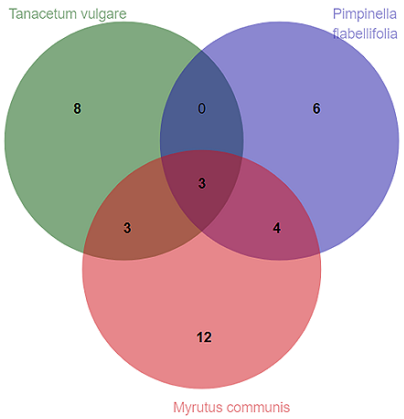

Figure 1. Venn diagram for the EO composition.

Table 2. Antioxidant properties of all three plants.

Sample	Inhibition IC50 (mg/mL) (DPPH)	Inhibition % (β-carotene-Linoleic acid)
Tanacetum vulgare	0.200±0.01	18.5±0.09
Pimpinella flabellifolia	0.083±0.003	57.2±2.18
Myrtus communis	0.500±0.085	37.07±1.25
BHT	0.0145±0.002	98.8±1.75

In an EO analysis study on Canadian varieties, the highest component was camphor at 30.48%, with the beta-thujone amount reported as 3.66% (29). In a similar study conducted on Poland species, the highest component was trans-chrysanthenyl acetate at 18.39%, while the beta-thujone amount was found to be 14.28% (15). Although the obtained content analysis results show regional variations, a common observation is that *T. vulgare* EO contains a high amount of beta-thujone. On the other hand, EO of *P. flabellifolia*, consisted of of (E)- anethol 48.30%, D-limonene 34.2% and (-)-α-Pinene 5.1% volatile compounds. Similar studies in the literature have consistently identified anethol and derivatives as the predominant component in the EO composition of this species (8). In the EO content of *M. communis*, a total of 93.19% has been identified, with high levels of volatile components, including 1,8-Cineole at 27.06%, (-)-α-Pinene at 14.94%, and linalool at 6.45%. A similar chemical profile was reported by Maharik et al., with the highest content being 1,8-Cineole at 31.98% and linalool at 21.94% (30). Considering the analysis of three essential oil samples, it was concluded that the chemical profile of the essential oil can vary significantly depending on the region from which it is collected, with *T. vulgare* identified as one such component.

Venn diagram analysis, conducted to uncover common component profiles and determine chemical synergy, revealed that the common component, alpha-pinene, was found in all three EO formulations, with the highest content determined in *M. communis* at 14.94%. Alpha-pinene, recognized as a high biotherapeutic product, has been reported to exhibit positive effects on the motor nervous system, in addition to its antimicrobial, antioxidant, and anti-inflammatory properties, particularly when exposed during the prenatal period in mice (31). In addition, regarding the commonality of chemical components, it has been concluded that *M. communis* and *P. flabellifolia* may exhibit volatile compound synergies with 7 common compounds (Figure 1).

Antioxidant Assays

Free radical DPPH and β-carotene-linoleic acid assay results for each EO samples were shown in Table 2. The antioxidant activities of EOs were analyzed using both DPPH and β-carotene assays. From the results, P. flabellifolia showed the highest antioxidant activity for both DPPH and β-carotene tests. Compared with the control groups, the plants exhibited a significantly high antioxidant profile, especially in the DPPH assays. The antioxidant properties obtained were found to be slightly lower than those of the T. vulgare species studied in Latvia (0.032-0.181 mg/mL depending on the plant parts) (32), and similar to those of the Romanian samples (33). On the other hand, antioxidant analyses for P. flabellifolia have been reported 0.00849 mg/mL for DPPH and 59.5% for β-carotene (8). It is possible that the species collected from the Erzincan/Turkey region exhibited antioxidant activity similar to the Sivas region. The antioxidant properties of M. communis were higher than those observed in previous studies conducted on M. communis EOs in Algeria, Tunisia, and Morocco, demonstrating IC50 values ranging between 0.80 and 4.50 mg/mL (34-36).

Table 3. Antimicrobial activities of the EOs using agar disc diffusion method.

Microorganisms	Disc Diffusiona Results			
	P. flabellifolia	***M. communis***	***T. vulgare***	**Gentamicin**
Staphylococcus aureus	18±1.01	29±1.00	16.5 ±0.50	23±0.54
Escherichia coli	11±0.26	18±0.45	3±0.04	16±0.20
Proteus vulgaris	11±1.93	10±1.70	-	22±1.45
Pseudomonas aeruginosa	6±1.01	6±1.01	84±2.04	20±0.28
Salmonella typhi	10±1.00	17±1.05	21±1.45	10±1.45
Bacillus subtilis	90±172	20±1.55	21±1.45	29±1.45
Klebsiella pneumoniae	60±1.81	23±1.62	16±0.88	20±1.45
Corynebacterium diphteriae	11±1.44	23±1.14	-	23±1.45
Candida albcans	38±1.47	15±1.47	12±1.45	-

Antimicrobial Assays

The antimicrobial properties of all EO samples were determined using the disc diffusion method, and the results are presented in Table 3.

The application of essential oils due to their antimicrobial activities is known in both animal husbandry and ethnoveterinary practices (37, 38) because bacterial and fungal infections in dairy cows can directly impact milk yield (39). Some findings have shown that these infections can be transmitted to humans if proper pasteurization processes are not ensured, such as E. coli contamination (40). On average, the antimicrobial activities were found to be in the following order, *P. flabellifolia* > *M. communis* > *T. vulgare*. In case of *P. flabellifolia*, results from the disc diffusion method indicate that *Bacillus subtilis* is the most sensitive microorganism, showing the highest inhibition zone, which is approximately three times larger than that of gentamicin. This followed by antimicrobial activity on *Pseudomonas aeruginosa* with *T. vulgare* EO. *P. aeruginosa* infections leading to mastitis are frequently observed in livestock, including animals raised in Türkiye (41) . The high activity of *T. vulgare* EO observed in the species could be considered a scientific basis for the traditional application of farmers administering the plant to cattle (38). Similarly, studies conducted on *Candida albicans*, a species causing mastitis in dairy cows, also confirm the high efficacy of Pimpinella EOs (42).

Molecular Docking Results

According to the results of in silico molecular docking analysis (Figure 2), alpha-terpinyl acetate (Pubchem ID: 111037) from *M. communis* and spathulenol (Pubchem ID: 92231) from *P. flabellifolia* exhibited the highest scores for both receptors.

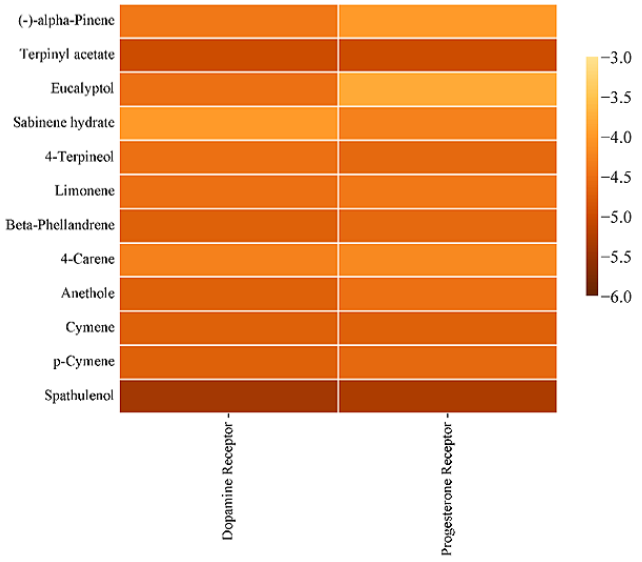

Figure 2. Clustered hierarchical heatmap showing molecular docking results.

Binding affinities for the dopamine receptor were -5.4 kcal/mol for alpha-terpinyl acetate and -5 kcal/mol for spathulenol (Figure 3). Likewise, for the progesterone receptor, the binding affinities were -5.3 kcal/mol for alpha-terpinyl acetate and -5 kcal/mol for spathulenol (Figure 4). For dopamine receptors, alpha-terpinyl acetate exhibited pi-sigma interactions at TYR71, formed a carbon-hydrogen bond at THR153, and engaged in alkyl interactions at VAL78, ILE156, and TRP160 residues. Additionally, Van der Waals interactions were observed at VAL74, SER75, and VAL152 residues. Conversely, spathulenol formed a carbon-hydrogen bond at THR153 and an alkyl interaction at ILE156. Furthermore, Van der Waals interactions occurred at TYR71, VAL74, SER75, VAL152, ALA157, and TRP160 residues.

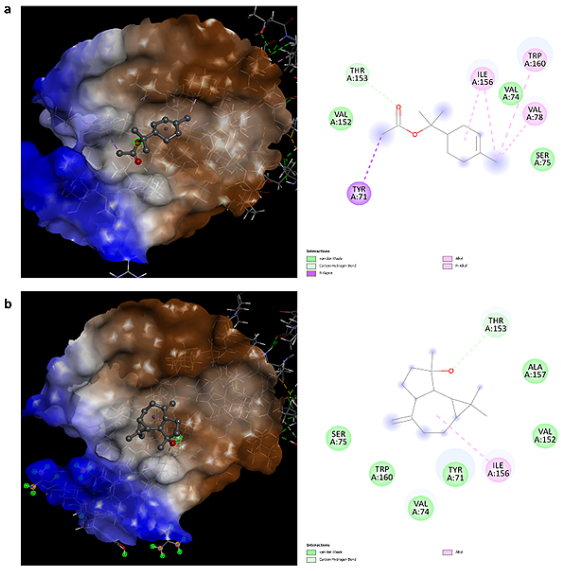

Figure 3. Dopamine receptor interactions (a) alpha-terpinyl acetate and (b) spathulenol.

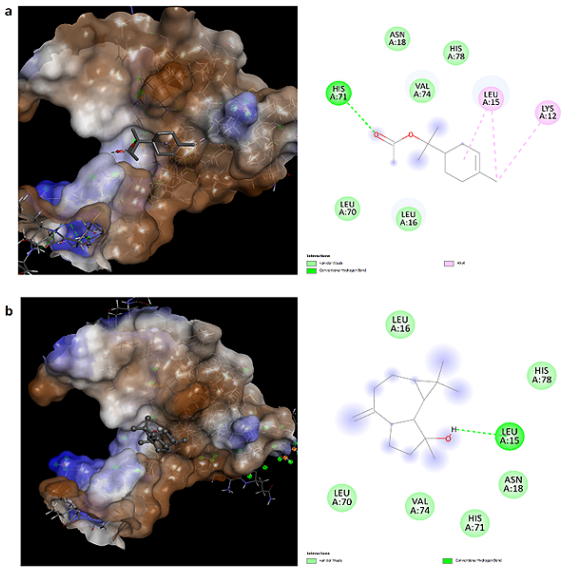

Figure 4. Progesterone receptor interactions (a) alpha-terpinyl acetate and (b) spathulenol.

Table 4. Carcinogenicity and mutagenicity prediction results.

	CarcinoPred -EL Method	Average	Predicted Result	Mutagenicity Prediction
T. vulgare Volatiles				
β-Thujone	XGBoost	0.59	Non-Carcinogen	non-mutagenic
beta-Cymene	XGBoost	0.36	Non-Carcinogen	non-mutagenic
bornyl acetate	XGBoost	0.49	Non-Carcinogen	non-mutagenic
P. flabellifolia Volatiles				
(E)- anethol	XGBoost	0.38	Non-Carcinogen	non-mutagenic
d-limonene	XGBoost	0.47	Non-Carcinogen	non-mutagenic
(-)-α-Pinene	XGBoost	0.48	Non-Carcinogen	non-mutagenic
M. communis Volatiles				
1,8-Cineole	XGBoost	0.48	Non-Carcinogen	non-mutagenic
linalool	XGBoost	0.39	Non-Carcinogen	non-mutagenic
thymol	XGBoost	0.46	Non-Carcinogen	non-mutagenic

On the other hand, when examining the interactions with progesterone receptors, it was observed that alpha-terpinyl acetate established conventional hydrogen bonds specifically at the HIS71 residue, engaging in alkyl interactions at both LYS12 and LEU15 residues. Additionally, this compound exhibited Van der Waals interactions with residues such as LEU16, ASN18, LEU70, VAL74, and HIS78. In stark contrast, spathulenol, another compound under scrutiny, demonstrated its ability to form conventional hydrogen bonds, primarily at the LEU15 residue, and also showed Van der Waals interactions with residues such as LEU16, ASN18, LEU70, VAL74, HIS71, and HIS78.

As depicted in Figures 3 and 4, the Bos taurus dopamine receptor demonstrates relative hydrophilicity at the binding pocket, whereas the progesterone receptor is even more hydrophilic than the dopamine receptor. This discrepancy may contribute to differences in the residue interaction types and distances for the same ligands. In addition, reference molecular docking analyses were conducted using dopamine for the dopamine receptor and progesterone for the progesterone receptor, yielding binding affinities of -4 kcal/mol and -6.8 kcal/mol, respectively. This comparative study highlights higher values obtained for the dopamine receptor, whereas the values obtained for the progesterone receptor are considered acceptable compared with those obtained for the progesterone molecule, which serves as the receptor's natural ligand. The results obtained from our analyses support the use of various parts of the *M. communis* plant as veterinary supplements for ewes, goats, and dairy cows for different purposes, which is consistent with the literature (43, 44). Considering the limited literature available due to its endemic nature, it is possible to establish the consistency of our results for *P. flabellifolia* by drawing parallels with other plant species within the Pimpinella genus used for similar purposes as veterinary supplements (10, 45).

Mutagenicity and Carcinogenicity Predictions

The computational analysis results regarding the probability of 3 highly abundant volatiles from the Eos being mutagenic and carcinogenic are presented in Table 4.

The average values calculated using the XGBoost method for highly carcinogenic compounds are close to 1, whereas the values obtained for these volatile compounds remain below the average carcinogenicity (0.5) (26). Most of these bioactive compounds, currently utilized in the pharmaceutical and cosmetic industries, as evident from the results, do not possess carcinogenic or mutagenic effects at moderate concentrations (46). In addition, based on predictions conducted on alpha-terpinyl acetate and spathulenol using data obtained from molecular docking results, no carcinogenic or mutagenic properties were detected.

ADMET Predictions

Estimation of various ADMET properties of the volatiles were given in the Table 5. LogP values measure the hydrophilicity or lipophilicity of a compound. Compounds with high logP values are categorized as lipophilic (hydrophobic), whereas those with low values are considered hydrophilic (47). Because all samples in the results are EOs, the volatile components are found moderately hydrophobic. These findings support the hypothesis that these components exhibit antimicrobial properties by disrupting the structure of bacterial cell membrane lipids (48). Upon examining the average logP values, it is evident that the values obtained for the three compounds, which constitute over 50% of the EO structure, demonstrate the highest hydrophobicity for *P. flabellifolia*. In addition, antimicrobial analysis results indicated that *P. flabellifolia* exhibited the highest activity. When evaluated in terms of BBB permeability, once again, *P. flabellifolia* has been found to exhibit high content and potency in terms of both

impact speed and strength. Research on other Pimpinella species regarding BBB permeability also emphasizes their rapid and potent efficacy (49). CNS permeability refers to the ability of a compound to penetrate the BBB and reach the tissues of the central nervous system. The average value of -2 obtained in the results indicates low CNS permeability (50). From this, it is understood that the application of essential oils carries a low probability of causing significant neurological effects or complications. Finally, it was observed that *P. flabellifolia* has the shortest clearance time among the applied essential oils. This suggests that the EO may remain in circulation for a longer period, potentially leading to an extended bioterapeutic effect.

Table 5. ADMET prediction results for three tested plants.

	LogP	Water solubility (log mol/L)	Intestinal absorption (%)	BBB permeability	CYP1A2 inhibitor	CNS permeability	Total Clearance (log ml/min/kg)	Hepatotoxicity
***T. vulgare* Volatiles**								
β-Thujone	2.2	-2.9	98.1	0.7	Yes	-1.9	0.13	No
beta-Cymene	3.1	-4	93.6	0.4	Yes	-1.3	0.2	No
bornyl acetate	2.7	-3.03	95.3	0.5	Yes	-2.3	1	No
***P. flabellifolia* Volatiles**								
(E)- anethol	2.7	-2.9	95.5	0.5	Yes	-1.6	0.2	No
d-limonene	3.3	-3.5	95.8	0.7	Yes	-2.3	0.2	No
(-)-α-Pinene	2.9	-3.7	96	0.7	Yes	-2.2	0.04	No
***M. communis* Volatiles**								
1,8-Cineole	2.7	-2.6	96.5	0.3	Yes	-2.9	1	No
linalool	2.6	-2.6	93.1	0.6	Yes	-2.3	0.5	No
thymol	2.8	-2.7	90.8	0.4	Yes	-1.6	0.2	Yes

Conclusion

This study aimed to assess the biological and biotherapeutic activities of the EOs from *Tanacetum vulgare* L., *Myrtus communis* L. subsp. communis L., and *Pimpinella flabellifolia* (Boiss.) Benth. Et Hook. ex Drude medicinal plants. GC/MS analysis results indicate that all three plants are rich in bioactive volatile components. According to computational prediction results, none of the components exhibit carcinogenicity or mutagenicity properties. Additionally, the antimicrobial results obtained, along with the ADMET properties, suggest that *Pimpinella flabellifolia* is the strongest plant in terms of biotherapeutic effect. Additionally, in silico analysis suggested that these plant species could be utilized as veterinary supplements for dairy cows, potentially enhancing lactation and overall well-being. Nevertheless, further studies are necessary to investigate in vitro and in vivo activity of lactating performance and other behaviors.

Declarations

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Conflict of Interest
The authors declare no conflicting interest.

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